Online Supplement
The Azithromycin for Acute Exacerbations of Asthma (AZALEA) Randomized Clinical Trial

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Supplementary methods

Study design

Visit 1 (day 1) occurred within 48 hours of initial presentation to medical care. Patients were then seen for Visits 2 (day 5±1 day, or exceptionally 2 days), 3 (day 10±1 day, or exceptionally 2 days) and 4 (day 42±2 weeks). At Visit 1 patients were instructed on symptom diary card recording and asked to complete the diary at the end of each day for 10 days. Symptom diary cards were reviewed at Visit 2 and 3. Convalescent serum was taken at Visit 4 for atypical bacterial serology.

We excluded subjects taking the following list of medications causing prolongation of the QT interval:

1. Amphetamines
2. Anti-emetics: Ondansetron, Dolasetron, Granisetron
3. Opioids: Methadone, Buprenorphine, Oxycodone
4. Antipsychotics: Droperidol, Thioridazine, Pimozide, Haloperidol, Chlorperazine
5. Antidepressants: Tricyclic Antidepressants, Trazodone
6. Antiarrhythmics: Quinidine, Disopyramide, Procainamide, Amiodarone, Sotalol
7. Antimalarials: Halofantrine
8. Cisapride
9. Cocaine

As there is no list that is regarded as definitive, this list was derived after consulting various different web-based sources and was agreed in consultation with all AZALEA PIs.

Bacteriology/Virology

We used in house PCR assays of nasal mucus samples, nasal and throat swabs and spontaneous or induced sputum to detect picornaviruses (mostly rhinoviruses); respiratory syncytial virus; coronaviruses 229E and OC43; parainfluenza viruses 1-3; influenza viruses AH1, AH3, and B; human metapneumoviruses; adenoviruses, bocavirus and the two atypical bacteria *Mycoplasma (M.) pneumoniae* and *Chlamydophila (C.) pneumoniae*, as described[1]. In addition we also used commercial MutaPLATE® real time (TaqMan) PCR kits for detection of *C. pneumoniae* and *M. pneumoniae* (Immundiagnostik, Bensheim, Germany) according to the manufacturer's instructions.

Serology for IgM for *M. pneumoniae* and *C. pneumoniae*, was performed on acute serum samples taken at exacerbation and for IgA and IgG for *M. pneumoniae* and *C. pneumoniae*, on acute serum samples taken at exacerbation and convalescent samples taken at Visit 4 using MEDAC *M. pneumoniae* and *C. pneumoniae* IgM, IgA and IgG ELISAs (Medac, Hamburg, Germany) according to the manufacturer's instructions.

All the above assays were performed centrally at Prof Johnston's laboratory at Imperial College London.

Standard sputum quantitative bacterial cultures were performed locally at each site using local Microbiology Laboratory standard operating procedures.

Statistical analyses

Sample Size

The sample size calculations were based on the primary outcome: the telithromycin study[2] found a mean difference in symptom score of -0.3 (standard deviation [SD] 0.783) between active and placebo groups at 10 days. Using a two-sided t-test at 1% significance level, with 80% power, 161 patients in each group were needed to detect the same difference in asthma scores between the groups. The significance level of 1% in the above calculation was chosen to provide greater certainty in assessment of the primary outcome variable and to provide greater power for the subgroup analyses. Assuming a drop-out rate of 15%[2], we proposed to recruit 190 patients to each arm.

Randomization was via a secure server performed using the InForm ITM (Integrated Trial Management) System. Patient allocation was stratified by center in random length blocks. The randomization lists were generated by an Imperial Clinical Trials Unit (ICTU) statistician. Details such as block size were kept confidential. There was no requirement for unblinding during the AZALEA trial therefore no patients were unblinded before statistical analysis.

Multilevel modeling: the three main components of the model

Let DS_{id} represent the diary score for patient *i* on day d, d = 1, …, 10, and *t(i)* represent the treatment given to individual *i* (azithromycin or placebo). Then model DS_{id} as the sum of three components: an intercept term, a change over time term and a residual error term, i.e.

$$DS_{id} = intercept_i + change\ over\ time_{t(i)d} + residual\ error_{id}$$

Possible choices for each of these components are outlined below. The options explored for the primary analysis were determined by the results of the exploratory analysis, and the final choice will be the simplest model that satisfies standard checks of model fit (e.g. residual plots).

Intercept term

The intercept term will estimate the diary score on day 1 (the day of randomization and start of the study medication). This term will comprise an individual level random effect, which will be drawn from a distribution parameterized using the associated center level random effect. Hence the unexplained variation in the diary scores will be split into three components corresponding to the three levels of the model, i.e. the variation attributable to the center (between center variation) and the individual (between individual variation), as well as the residual variation (within individual variation).

Additionally, baseline covariates can be incorporated into the model at the individual level. None will be incorporated for the initial analysis unless the baseline characteristics analysis reveals a substantial imbalance. Further analyses will examine the effect of incorporating baseline variables (age, gender, asthma severity, smoking history and asthma exacerbation).

Change over time (cot) term

This term will capture the change in the diary score from the start of the study medication (day 1), hence time will enter the model as day 1. The simplest assumption would be a linear change over the period, however alternatives may need to be considered as the rate of change may not be constant over the 10 day period. Alternatives are to include a quadratic term or use splines. The coefficients in this term will be dependent upon treatment.

Residual error term

We were assuming that the residual errors have a Normal distribution. An alternative was to assume that these errors follow a heavier tailed distribution such as a t distribution with 4 degree of freedom, which will provide robustness to outliers. Normality of residual error was checked graphically.

Missing data

Before starting data analysis, the level and pattern of the missing data in the baseline variables and outcomes was analyzed by forming appropriate tables. Additionally, the likely causes of any omissions were investigated. This information was used to determine whether the level and type of missing data had the potential to introduce bias into the analysis or to substantially reduce the precision of estimates related to treatment effects. Missing data in the patient diary took one of several forms: no patient diary returned for any day (patient omissions), all data missing for one or more days (day omissions) and data missing for some but not all the individual questions for a particular day (item omissions). Of these, the level of item omissions was expected to be minimal. According to the SAP if any item omissions occurred in diary scores, the scores for the missing questions were interpolated from the previous and subsequent day scores. This process was conducted for 2 missing entries.
If any item omissions occurred in AQLQ scores the summary score for that day was treated as missing.
Missing data for the pulmonary function tests were expected to be due to the spirometer not recording some measures. As this was unrelated to the patient outcome, it was reasonable to assume that these omissions were uninformative and that multi-level models fitted to all observed data would provide unbiased parameter estimates.

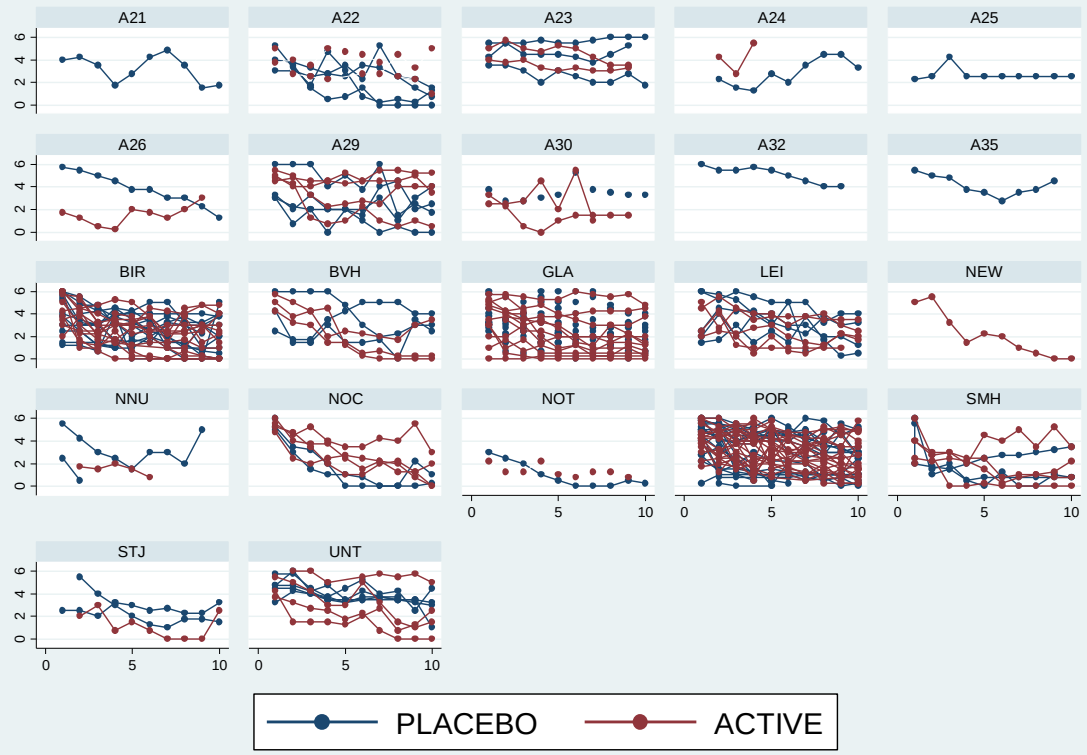
Supplementary results

Of the 199 patients randomized, 193 (97%) were from secondary care hospitals and 6 (3%) from the primary care center.

Exploratory analysis of the primary outcome

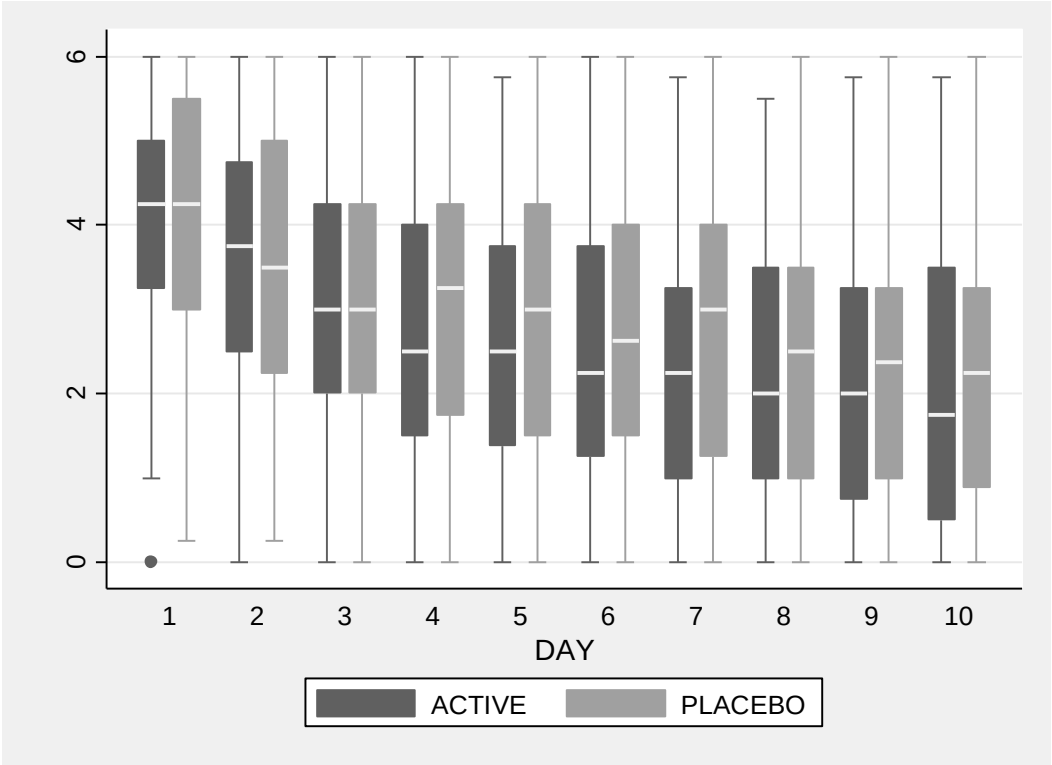
As a check for outliers and imbalances, a series of longitudinal plots (one for each center) of diary score for each patient, differentiating between treatment arm were produced (see **Supplementary eFigure 1**). Boxplots of diary scores by treatment arm for each day were produced to show the distribution of the observed scores graphically in **Supplementary eFigure 2**. **Supplementary eTable 1** shows the observed mean diary scores and standard deviations for each treatment arm by day and the number of observations. Additionally, a table of summary statistics of the diary scores by day and treatment arm was produced, including the number of observations, mean, standard deviation, median, lower and upper quartiles (**Supplementary eTable 2**).

Supplementary eFigure 1: Observed diary scores for each center by treatment arm



A21	Royal Berkshire Hospital
A22	Rowden Surgery
A23	East Surrey Hospital
A24	Countess of Chester
A25	Musgrove Park Hospital
A26	Worcester Acute Hospital
A29	New Cross Hospital, Royal Wolverhampton
A30	Ipswich Hospital, NHS Trust
A32	Telford
A35	Gloucestershire Royal Hospital
BIR	Heart of England NHS Foundation Trust
BVH	Blackpool Victoria Hospital
GLA	Western Infirmary Glasgow
LEI	University Hospitals of Leicester NHS Trust
NEW	The Newcastle upon Tyne Hospitals NHS Foundation Trust
NNU	Norfolk and Norwich University Hospital
NOC	Nottingham City Hospital
NOT	Queen's Medical Centre, Nottingham
POR	Portsmouth Hospitals NHS Trust
SMH	St Mary's Hospital, Imperial College Healthcare NHS Trust
STJ	St James's University Hospital
UNT	University Hospital of North Tees

Supplementary eFigure 2: Boxplots of observed symptom diary scores



Supplementary eTable 1: Observed mean symptom scores for each day by treatment group and their standard deviation

	day 1	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**
Placebo (SD)	4.18 (1.48)	3.45 (1.62)	3.12 (1.47)	3.04 (1.57)	2.87 (1.58)	2.79 (1.56)	2.80 (1.69)	2.43 (1.53)	2.32 (1.55)	2.20 (1.51)
N	77	86	85	81	81	80	79	77	74	68
Active (SD)	4.14 (1.38)	3.51 (1.42)	3.09 (1.45)	2.78 (1.58)	2.63 (1.51)	2.44 (1.54)	2.19 (1.53)	2.24 (1.61)	2.22 (1.71)	2.09 (1.71)
N	71	85	86	84	80	78	81	80	78	71

Supplementary eTable 2: Detailed statistics of observed diary scores

Placebo				Active			
	N	**Diary score, mean (SD)**	**Diary score, median (IQR)**		**N**	**Diary score, mean (SD)**	**Diary score, median (IQR)**
day 1	77	4.18 (1.48)	4.25 (3.00, 5.50)	**day 1**	71	4.14 (1.38)	4.25 (3.25, 5.00)
day 2	86	3.45 (1.62)	3.50 (2.25, 5.00)	**day 2**	85	3.51 (1.42)	3.75 (2.50, 4.75)
day 3	85	3.12 (1.47)	3.00 (2.00, 4.25)	**day 3**	86	3.09 (1.45)	3.00 (2.00, 4.25)
day 4	81	3.04 (1.57)	3.25 (1.75, 4.25)	**day 4**	84	2.78 (1.58)	2.50 (1.50, 4.00)
day 5	81	2.87 (1.58)	3.00 (1.50, 4.25)	**day 5**	80	2.63 (1.51)	2.50 (1.38, 3.75)
day 6	80	2.79 (1.56)	2.63 (1.50, 4.00)	**day 6**	78	2.44 (1.54)	2.25 (1.25, 3.75)
day 7	79	2.80 (1.69)	3.00 (1.25, 4.00)	**day 7**	81	2.19 (1.53)	2.25 (1.00, 3.25)
day 8	77	2.43 (1.53)	2.50 (1.00, 3.50)	**day 8**	80	2.24 (1.61)	2.00 (1.00, 3.50)
day 9	74	2.32 (1.55)	2.38 (1.00, 3.25)	**day 9**	78	2.22 (1.71)	2.00 (0.75, 3.25)
day 10	68	2.20 (1.51)	2.25 (0.88, 3.25)	**day 10**	71	2.09 (1.71)	1.75 (0.50, 3.50)

A linear change was assumed in the model for the diary score over time with different slopes for the two treatment arms. Additionally, equal mean scores were assumed at baseline for the two groups as any inequality could only have occurred by chance, due to randomization. In order to reduce bias caused by the observed difference at baseline, the main effect of the interaction term was not included in the model as an independent covariate. Sensitivity analysis with the inclusion of this covariate was conducted. The estimated mean diary score at baseline (day 1) in the whole study population was 3.66 (95% CI: 3.41; 3.90). In addition to the decrease observed in the placebo group, the decrease of the diary score in the azithromycin group was slightly greater. On average the difference in change compared to the placebo group was -0.018 per day (95% CI: -0.074 , 0.037). The estimated differences with their 95% confidence intervals for each day can be found in **Supplementary eTable 3**. The mean "natural" background daily decrease (decrease in placebo group) in diary score was -0.18 (95% CI for the first day alone: -0.22; 0.14). On day 10, the difference between the two groups was not statistically significant. The estimated mean diary score was lower in the azithromycin group by -0.166 (95% CI: -0.670; 0.337). On Day 5 the difference was -0.074 (95% CI: -0.298; 0.150) between the two groups.

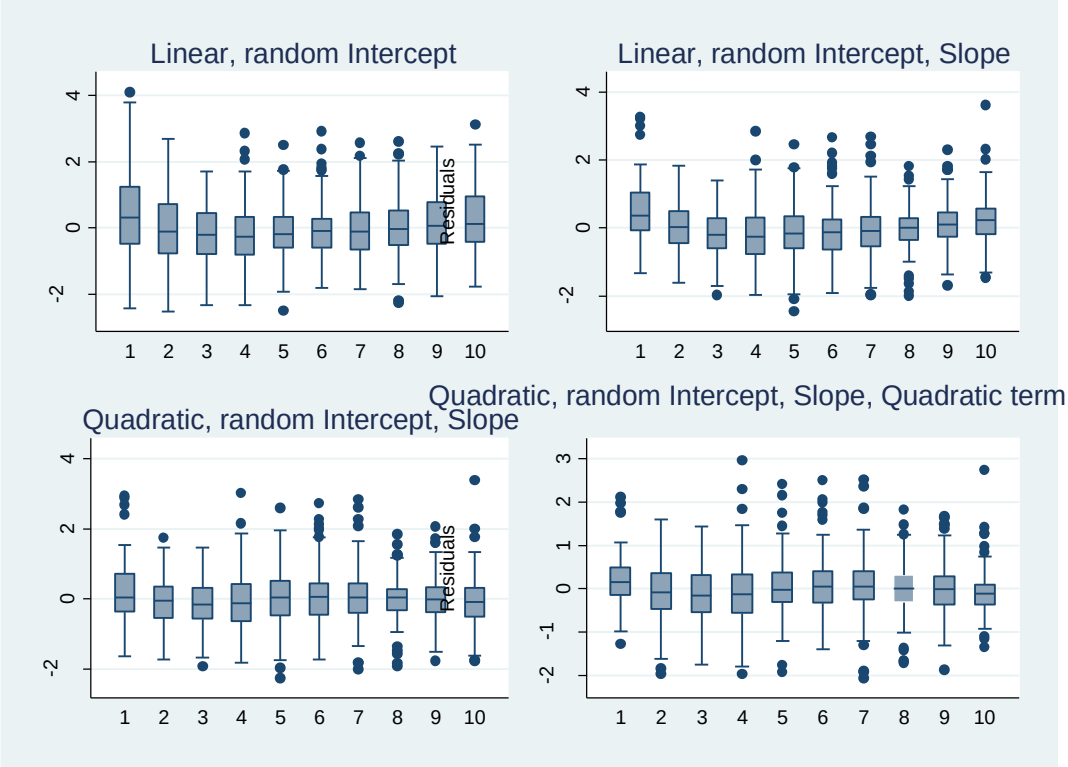
Supplementary eTable 3: Estimated difference in change of diary scores from baseline and 95% confidence intervals for azithromycin compared to the placebo

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
Difference in Change from baseline	0	-0.018	-0.037	-0.055	-0.074	-0.092	-0.111	-0.129	-0.148	**-0.166**
95% Confidence Interval	- -	-0.074 0.037	-0.149 0.075	-0.223 0.112	-0.298 0.150	-0.372 0.187	-0.446 0.224	-0.521 0.262	-0.595 0.299	**-0.670** **0.337**

Model selection

Different relationships between time and diary scores were compared including linear, quadratic and square root relationships. These models differed in their ”time” covariate. Fixed and random effects and the use of splines were also investigated. The plots of level 1 and level 2 residuals (where appropriate) were assessed for these models, including the model with splines at day 3 and day 7 and the fitted and observed values were also investigated graphically. As it can be seen (**Supplementary eFigure 3**), the more complex alternative models gave more flexibility than the standard linear model, but overall the residuals were just barely lower and the pattern of residuals remain the same, so in order of simplicity a linear model was chosen to calculate the estimated scores.

Supplementary eFigure 3: Boxplot of residuals for linear and quadratic models



Details of the models for diary and AQLQ Scores

Supplementary eTable 4: Diary score

Fixed-effects Parameters				
Covariates		Coefficient	95% CI	P value
Constant	Mean score at baseline in the Placebo group	3.6595	3.4169 3.9022	0.000
Days (centered)	Daily change in Placebo group	-0.1792	-0.2217 -0.1367	0.000
Treatment #Day (interaction)	(Treatment effect) Difference in daily change compared to the Placebo group	-0.0185	-0.0744 0.0374	0.517

Random-effects Parameters			
Level	variance	Estimate	95% CI*
Site	Constant (intercept)	0.0412	0.0012 1.4372
Subject	Constant (intercept) Days (slope)	1.6863 0.0334	1.3063 2.1769 0.0251 0.0443
	Covariance Days - Constant	-0.0957	-0.1461 -0.0453
	residuals	0.6941	0.6415 0.7510

*****95% confidence intervals presented for the variance parameters should not be used to test the significance of the variance parameters as the lower limits of these intervals can never be smaller than zero since variances are strictly positive quantities

LR test vs. linear regression: $p < 0.0001$

Supplementary eTable 5: Acute AQLQ

Fixed-effects Parameters				
Covariates		Coefficient	95% CI	P value
Constant	Mean score at baseline in the Placebo group	4.727	4.491 4.962	0.000
Visits (centered)	Per visit change in Placebo group	0.429	0.275 0.583	0.000
Treatment #Visit (interaction)	(Treatment effect)	0.065	-0.138 0.269	0.530

Random-effects Parameters			
Level	variance	Estimate	95% CI*
Site	Constant (intercept)	0.063	0.009 0.450
Subject	Constant (intercept) Visits (slope)	0.888 0.165	0.583 1.353 0.059 0.464
	Covariance Visits - Constant	-0.074	-0.272 0.125
	residuals	0.903	0.727 1.123

*****95% confidence intervals presented for the variance parameters should not be used to test the significance of the variance parameters as the lower limits of these intervals can never be smaller than zero since variances are strictly positive quantities

Supplementary eTable 6: Mini AQLQ

Fixed-effects Parameters				
Covariates		Coefficient	95% CI	P value
Constant	Mean score at baseline in the Placebo group	3.355	3.196 3.514	0.000
Visits (centered)	Per visit change in Placebo group	0.350	0.214 0.486	0.000
Treatment #Visit (interaction)	(Treatment effect)	-0.021	-0.204 0.163	0.823

Random-effects Parameters			
Level	variance	Estimate	95% CI*
Site	Constant (intercept)	0.000	0.000 0.000
Subject	Constant (intercept) Visits (slope)	0.803 0.185	0.569 1.133 0.097 0.350
	Covariance Visits - Constant	-0.076	-0.220 0.069
	residuals	0.566	0.457 0.703

*****95% confidence intervals presented for the variance parameters should not be used to test the significance of the variance parameters as the lower limits of these intervals can never be smaller than zero since variances are strictly positive quantities

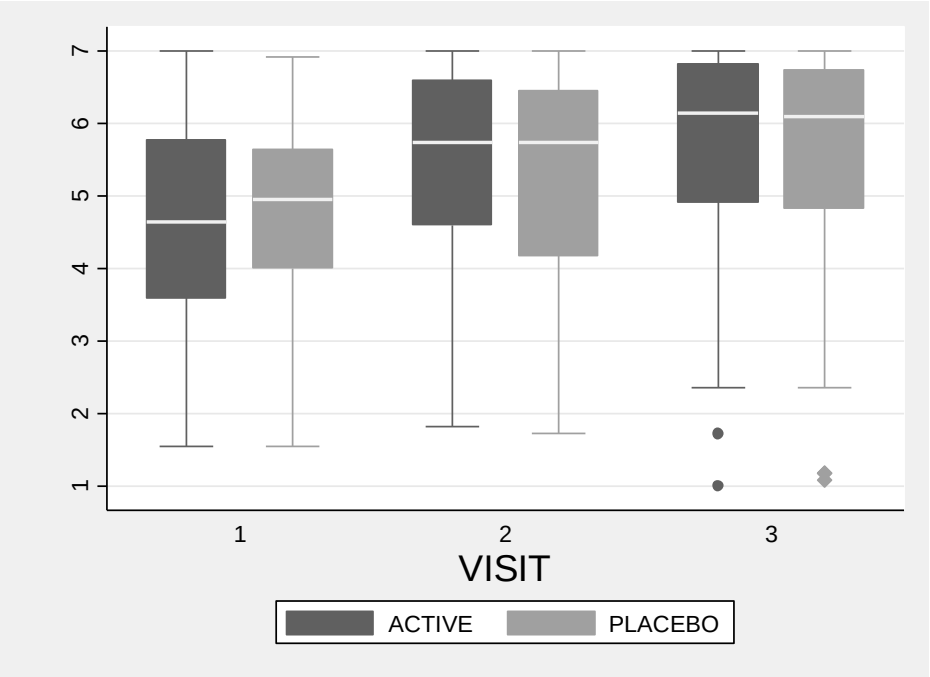
Secondary outcome analysis

For all secondary outcomes, an exploratory analysis and assessment of missing data was completed prior to the main analysis. This was analogous to that outlined for the primary outcome. Multilevel models, similar to those specified for the primary outcome, were used to analyze the acute asthma and mini-asthma questionnaires and also for the pulmonary function tests. Details of the models used for AQLQ and mini AQLQ respectively can be found in **Supplementary eTables 5 and 6**.

Acute AQLQ and mini AQLQ analysis

Boxplots of acute AQLQ by treatment arm for each visit are shown in **Supplementary eFigure 4**.

Supplementary eFigure 4: Boxplots of observed acute AQLQ scores



Supplementary eTable 7 shows the observed mean and standard deviation of Acute AQLQ scores for each treatment arm by visit and the number of observations.

Supplementary eTable 7: Detailed statistics of observed acute AQLQ scores

Placebo						
		Acute AQLQ				
Visit	**N**	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	100	4.8	1.3	5.0	4.0	5.6
2	87	5.3	1.4	5.7	4.2	6.4
3	83	5.6	1.5	6.1	4.8	6.7
Active						
		Acute AQLQ				
Visit	**N**	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	96	4.6	1.4	4.6	3.6	5.8
2	84	5.4	1.3	5.7	4.6	6.6
3	80	5.6	1.5	6.1	4.9	6.8

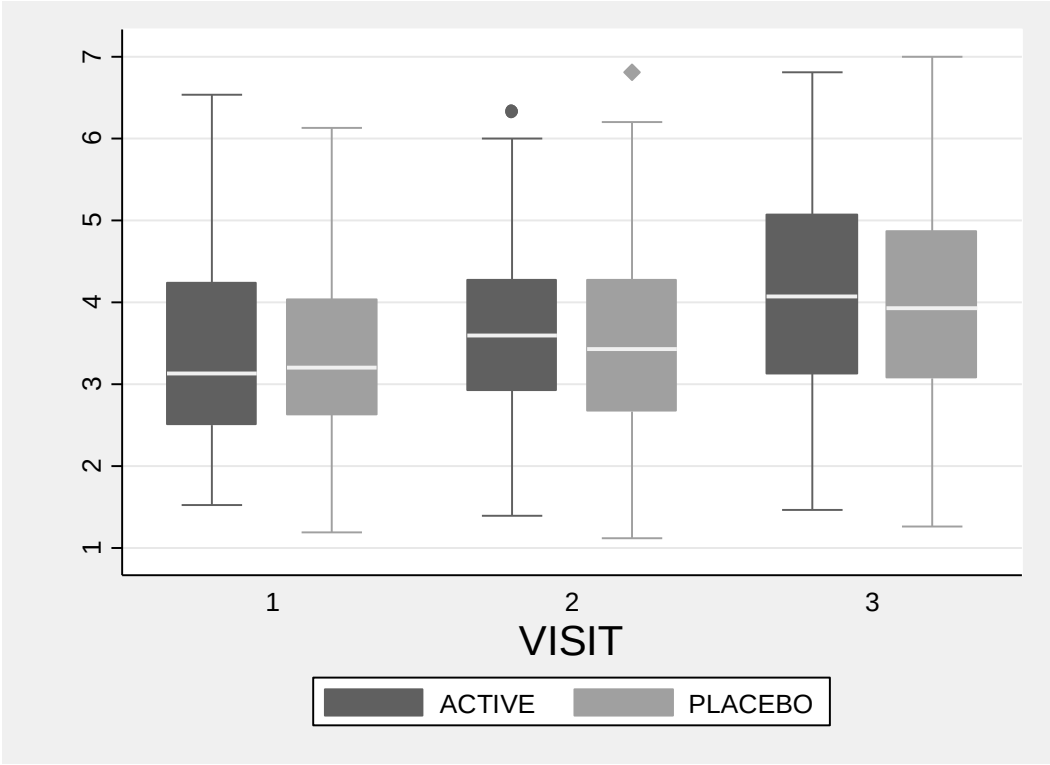
As for the primary outcome, multilevel modeling was carried out assuming equal mean scores at baseline and linear change for the acute AQLQ and mini-AQLQ scores over time with different slopes for the two treatment arms. Differences in the change of acute AQLQ scores for each visit with the 95% confidence intervals can be found in **Supplementary eTable 8**. At visit 3 (day 10) there was no statistically significant difference between the two groups. According to the model, at visit 3, there was 0.130 (95% CI: -0.276; 0.539) greater acute AQLQ score estimated in the azithromycin group than the placebo group.

Supplementary eTable 8: Estimated difference in acute AQLQ score by visits

Acute AQLQ score	**Visit 1 (Day 1)**	**Visit 2 (Day 5)**	**Visit 3 (Day 10)**
Difference in change compared to Placebo group	0	0.065	0.130
95% Confidence Interval	-	-0.138; 0.269	-0.276; 0.539

The same analyses were conducted for mini AQLQ scores as for acute AQLQ scores. Boxplots of Mini-AQLQ, by treatment arm, for each visit, are shown in **Supplementary eFigure 5**. **Supplementary eTable 9** shows the observed mean and standard deviation of mini AQLQ scores for each treatment arm by visit.

Supplementary eFigure 5: Boxplots of observed mini AQLQ scores



Supplementary eTable 9: Detailed statistics of observed mini AQLQ scores

Placebo					
	Mini AQLQ				
Visit	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	3.4	1.1	3.2	2.6	4.0
2	3.6	1.2	3.4	2.7	4.3
3	4.1	1.3	3.9	3.1	4.9
Azithromycin					
	Mini AQLQ				
Visit	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	3.4	1.2	3.1	2.5	4.2
2	3.6	1.1	3.6	2.9	4.3
3	4.1	1.3	4.1	3.1	5.1

Differences in the change of mini AQLQ scores for each visit with 95% confidence intervals are shown in **Supplementary eTable 10**. At visit 3 (day 10) there was no statistically significant difference between the two groups. According to the model, at visit 3 there was -0.042 (95% CI: -0.409; 0.325) lower mini AQLQ score estimated in the azithromycin group than the placebo group..

Supplementary eTable 10: Estimated difference in mini AQLQ score azithromycin compared to placebo by visits

Mini AQLQ	Visit 1 (Day 1)	Visit 2 (Day 5)	Visit 3 (Day 10)
Difference in change compared to Placebo group	0	-0.020	-0.042
95% Confidence interval	-	-0.204; 0.163	-0.409; 0.325

Pulmonary function test analysis

For the pulmonary function tests similar exploratory analyses and multilevel modelling was conducted as for AQLQ scores. **Supplementary eTable 11** shows the observed pulmonary function test values (mean and standard error) for each visit by treatment arm.
Supplementary eTable 12 shows the estimated differences in change for azithromycin compared to placebo group with 95% confidence intervals by visit for each pulmonary function test.

Supplementary eTable 11: Observed mean (SD) pulmonary function test results by visit and treatment arm

Active Group				**Placebo group**		
Visit 1 Day 1	**Visit 2 Day 5**	**Visit 3 Day 10**		**Visit 1 Day 1**	**Visit 2 Day 5**	**Visit 3 Day 10**
97	85	80	N	101	90	83
1.94 (0.74)	2.23 (0.77)	2.30 (0.83)	**FEV_1(liters), mean (SD)**	2.11 (0.79)	2.34 (0.83)	2.38 (0.91)
2.80 (1.03)	3.13 (1.00)	3.25 (1.08)	**FVC(liters), mean (SD)**	3.09 (1.05)	3.40 (1.10)	3.38 (1.09)
69.66 (13.33)	71.71 (12.02)	71.00 (12.38)	**FEV_1/FVC ratio, mean (SD)**	68.83 (13.71)	69.28 (12.24)	70.02 (12.71)
1.59 (0.89)	1.85 (0.94)	1.77 (0.92)	**$FEF_{25-75\%}$(liters/sec), mean (SD)**	1.74 (1.14)	1.83 (1.08)	1.94 (1.20)
1.92 (1.06)	2.12 (1.05)	2.19 (1.08)	**$FEF_{50\%}$(liters/sec), mean (SD)**	2.04 (1.26)	2.15 (1.24)	2.32 (1.35)
288.0 (107.5)	345.0 (109.0)	363.3 (108.4)	**PEF(liters/min), mean (SD)**	320.2 (102.6)	349.5 (110.1)	356.8 (118.1)

Supplementary eTable 12: Estimates of pulmonary function mean differences and 95% CI in brackets

	Difference in change compared to Placebo at visit 3 (Day 10)	Difference in change compared to Placebo at visit 2 (Day 5)	Per visit change in Placebo	Baseline mean
FEV_1(liters)	0.050 (-0.132; 0.231)	0.024 (-0.067; 0.116)	0.164 (0.099; 0.228)	2.011 (1.875; 2.146)
FVC(liters)	0.038 (-0.166; 0.243)	0.019 (-0.083; 0.122)	0.200 (0.127; 0.272)	2.959 (2.809; 3.110)
FEV_1/FVC ratio	1.379 (-1.559; 4.316)	0.689 (-0.779; 2.158)	0.365 (-0.732; 1.463)	69.5 (67.7; 71.4)
$FEF_{25-75\%}$(liters/sec)	0.036 (-0.192; 0.265)	0.018 (-0.096; 0.132)	0.116 (0.035; 0.197)	1.631 (1.470; 1.792)
$FEF_{50\%}$(liters/sec)	0.045 (-0.234; 0.324)	0.022 (-0.117; 0.162)	0.161 (0.062; 0.260)	1.931 (1.750; 2.112)
PEF(liters/min)	18.03 (-8.56; 44.62)	9.016 (-4.278; 22.31)	24.66 (15.01; 34.31)	296.3 (272.0; 321.6)

Subgroup studies

The same model as outlined for the primary outcome was used for subgroup analyses which including the following:

- Bacteria culture positive or negative in sputum: **Supplementary eTable 13**
- Viral tests positive or negative in nasal mucus, nasal swab, throat swab or sputum: **Supplementary eTable 14**
- Atypical bacteria positive or negative in nasal mucus, nasal swab, throat swab, sputum or serological testing: **Supplementary eTable 15**

Sputum bacterial culture was positive in 6% of subjects (4.1% active, 7.8% placebo). Nasal/throat swab/mucus and/or sputum atypical bacterial PCR and/or atypical bacterial serology were positive in 4.5% of patients (5.2% active, 3.9% placebo). Overall a bacteria/atypical bacterial test positive occurred in 10.6% of patients (9.3% active, 11.8% placebo). Nasal/throat swab/mucus and/or sputum virus PCR were positive in 18.1% of patients (16.5% active, 19.6% placebo).

Supplementary eTable 13: Estimated Day 10 difference in change of diary scores from baseline with 95% confidence intervals with azithromycin compared to the placebo group in sputum culture bacteria positive or negative subgroup

Group	Whole study population (N=176)	Sputum bacterial culture missing (N=93)	Sputum bacterial culture positive (N= 12)	Sputum bacterial culture negative (N= 71)
Day 10 difference in change	-0.166	-0.114	1.178	-0.410
95% CI	-0.670; 0.337	-0.821; 0.594	-0.497; 2.853	-1.183; 0.364

Supplementary eTable 14: Estimated Day 10 difference in change of diary scores from baseline with 95% confidence intervals for azithromycin compared to placebo in virus PCR test positive or negative subgroups

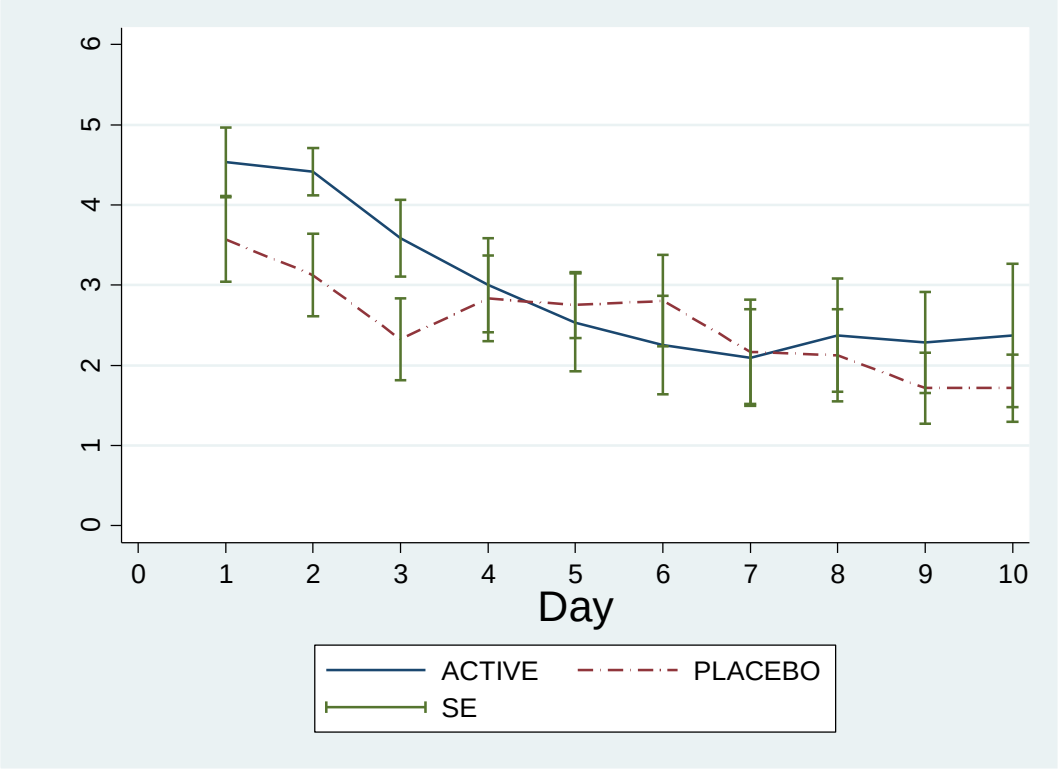
Group	Whole study population (N=176)	Virus PCR positive (N=31)	Virus PCR negative (N= 138)
Day 10 difference in change	-0.166	-0.100	*-0.106*
95% CI	-0.670; 0.337	-1.170; 0.969	*-0.683; 0.472*

Supplementary eTable 15: Estimated Day 10 difference in change of diary scores from baseline with 95% confidence intervals for azithromycin compared to the placebo group in atypical bacteria and any bacteria positive or negative subgroups

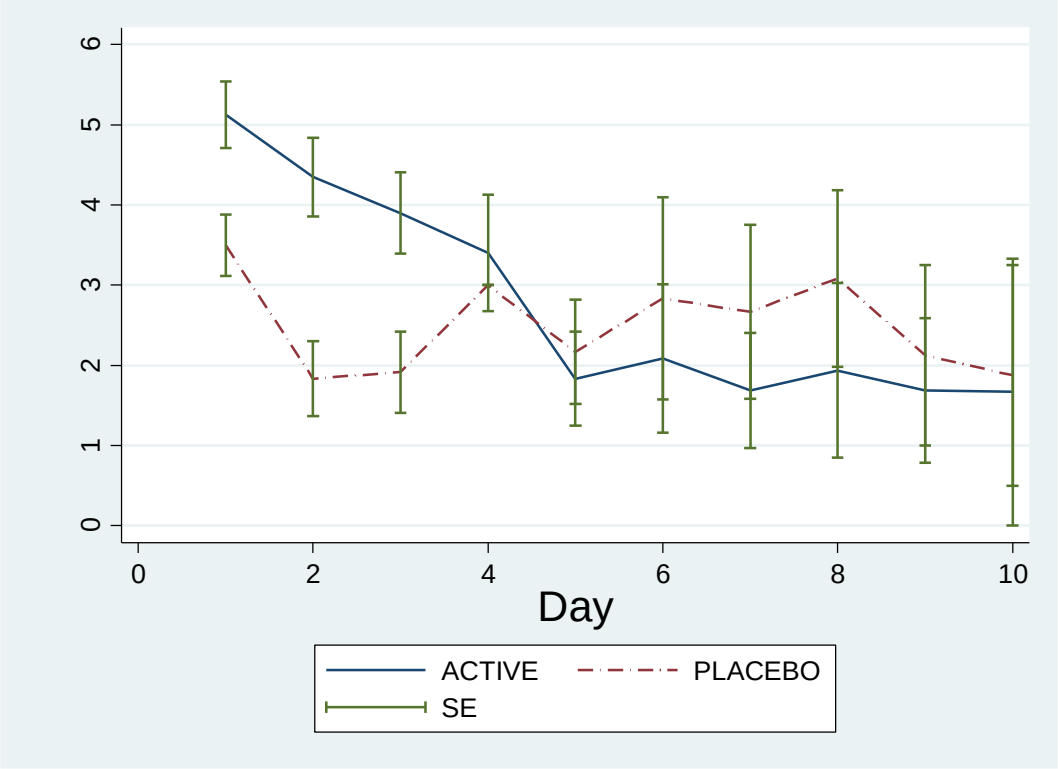
Group	Whole study population (N=176)	Atypical* bacteria positive (N=8†)	Atypical* bacteria negative (N=157)	Any bacterial test positive (N=20)
Day 10 difference in change	-0.166	1.391	0.044	0.198
95% CI	-0.670; 0.337	-1.214; 3.996	-0.465; 0.554	-1.546; 1.942

* *C. pneumoniae* or *M. pneumoniae*
†There were 9 patients with positive atypical bacteriology test results, but one of them had no diary score records

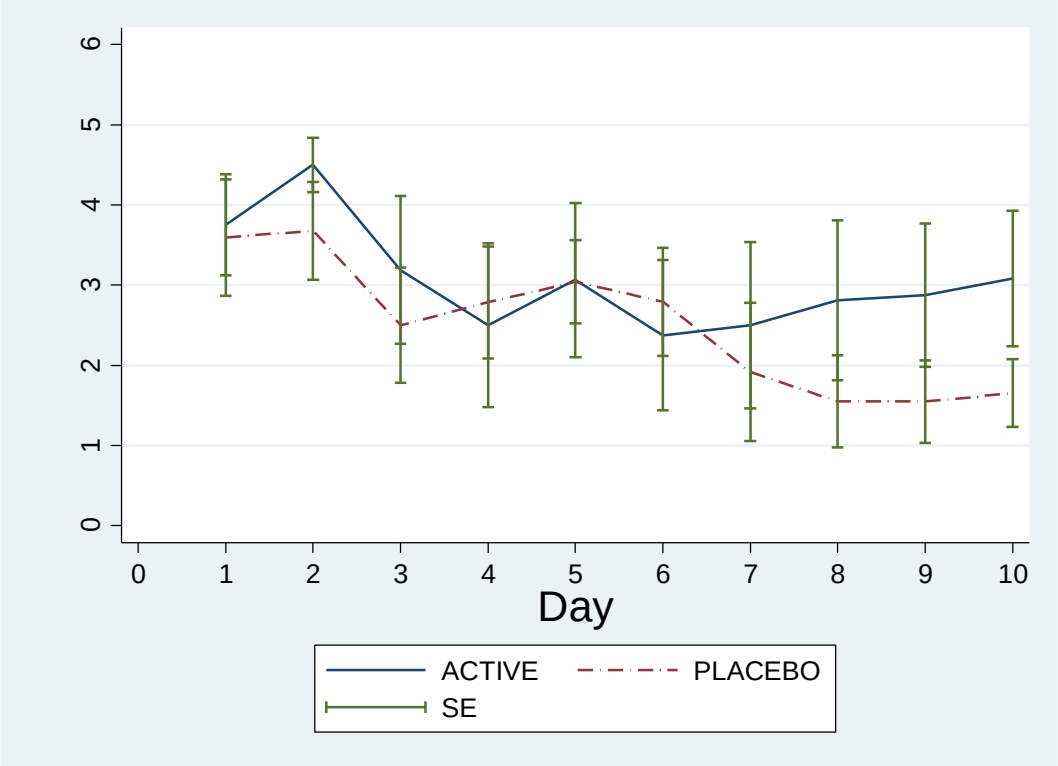
Supplementary eFigure 6: Observed mean diary scores and standard errors of the any bacterial test positive subgroup (N=20) by treatment arm



Supplementary eFigure 7: Observed mean diary scores and standard errors of the atypical bacterial test positive subgroup (N=8) by treatment arm



Supplementary eFigure 8: Observed mean diary scores and standard errors of the Bacteria culture positive in sputum subgroup (N=12) by treatment arm



Subgroup analysis based on time to receipt of study drug
A subgroup analysis on those who received study drug within 24hrs of initial presentation to medical care (N=104, 52 azithromycin, 52 placebo, difference at day 10 0.001 (95% CI: -0.634 to 0.636) and those who received study drug 24hrs or more after initial presentation (N=72, 35 azithromycin, 37 placebo, difference at day 10 -0.356 (95% CI: -1.128 to -0.417) suggested there was no evidence that benefit may have been greater in those that received study drug earlier.

Post Hoc analysis
The AZISAST study reported that azithromycin prophylaxis reduced exacerbations in subjects with non-eosinophilic severe asthma (blood eosinophilia ≤200/μL): 0.44 (95% CI 0.25 to 0.78) versus 1.03 (95% CI 0.72 to 1.48) (P=0.013)[3]. We therefore carried out a similar post hoc analysis. There were 166 patients with blood eosinophil results and diary score records. Multilevel modeling of our primary outcome revealed no significant benefit in those with blood eosinophils <200/μL (N=103: difference -0.265; 95% CI -0.873 to 0.363) or <300/μL (N=118: difference: -0.180; 95% CI -0.791 to 0.432).

Safety data analysis

Protocol reporting of adverse events were from the time the patient gave informed consent until seven days after the last dose of study medication. Using the information recorded on the adverse event eCRF, each adverse event was categorized using MedDRA coding System Organ Class (SOC) terms by a designee of the Chief Investigator. The number of adverse events and patients affected in each category by treatment arm can be found in **Supplementary eTable 16** and **Supplementary eTable 17**.

Supplementary eTable 1: Number of adverse events by SOC category and treatment arm

	Arm		
Adverse Event Category*	Active	Placebo	Total
	No.	No.	No.
Cardiac disorders	4	2	6
Eye disorders	2	1	3
Gastrointestinal disorders	35	24	59
General disorders	18	25	43
Infections and infestations	0	1	1
Musculoskeletal and connective tissue disorders	4	6	10
Nervous system disorders	15	14	29
Psychiatric disorders	1	2	3
Reproductive system and breast disorders	0	1	1
Respiratory, thoracic and mediastinal disorders	27	37	64
Skin and subcutaneous disorders	0	1	1
Total	106	114	220

*as advised by Chief Investigator or designee, based on description

Supplementary eTable 17: Number of patients affected by SOC category (a patient is only shown once in each category)

	Arm		
Adverse Event Category*	**Active**	**Placebo**	**Total**
	No.	**No.**	**No.**
Cardiac disorders	4	2	6
Eye disorders	2	1	3
Gastrointestinal disorders	25	20	45
General disorders	16	19	35
Infections and infestations	0	1	1
Musculoskeletal and connective tissue disorders	3	4	7
Nervous system disorders	14	13	27
Psychiatric disorders	1	2	3
Reproductive system and breast disorders	0	1	1
Respiratory, thoracic and mediastinal disorders	20	28	48
Skin and subcutaneous disorders	0	1	1
Total† (number of patients affected)	**85(51)**	**92 (52)**	**177 (103)**

*as advised by Chief Investigator or designee, based on description
†a patient may have more than one adverse event in any category

Supplementary eTable 18 shows the number of adverse events by category and relationship to study medication. The relationship is missing for four adverse events, and these are shown as "Unknown". No adverse events were definitely related to the study medication.

Supplementary eTable 18: Number of Adverse Events by SOC category and Relationship to Study Medication

	Relationship to study Medication					
Adverse Event Category*	**Not related**	**Unlikely**	**Possible**	**Probable**	**Unknown**	**Total**
	No.	**No.**	**No.**	**No.**	**No.**	**No.**
Cardiac disorders	3	2	1	0	0	6
Eye disorders	1	2	0	0	0	3
Gastrointestinal disorders	9	5	36	7	2	59
General disorders	20	11	11	0	1	43
Infections and infestations	1	0	0	0	0	1
Musculoskeletal and connective tissue disorders	6	3	1	0	0	10
Nervous system disorders	8	13	8	0	0	29
Psychiatric disorders	0	3	0	0	0	3
Reproductive system and breast disorders	1	0	0	0	0	1
Respiratory, thoracic and mediastinal disorders	49	14	0	0	1	64
Skin and subcutaneous disorders	0	0	1	0	0	1
Total	**98**	**53**	**58**	**7**	**4**	**220**

*as advised by Chief Investigator or designee, based on description

Multiple adverse events were reported for some patients, with 51 patients (just less than half of those with adverse events) reporting more than one. Ten adverse events were reported for one subject. **Supplementary eTable 19** provides further detail about the distribution of the 220 adverse events between the 103 patients who reported adverse events.

Supplementary eTable 19: Number of Adverse Events Reported for Individual patients

	Treatment Arm		
Number of Adverse Events	**Active**	**Placebo**	**Total**
	No.	**No.**	**No.**
1	24	28	52
2	12	9	21
3	7	6	13
4	4	4	8
5	3	2	5
6	1	1	2
8	0	1	1
10	0	1	1
Total	**51**	**52**	**103**

Details of the adverse events classified as cardiac disorders are given in **Supplementary eTable 20**. None of these were classified as a serious adverse event.

Supplementary eTable 20: Listing of adverse events classified as Cardiac Disorders

Age (years)	Arm	Description	Site*	Relation	Severity	Outcome	Action †	Duration
26	PLACEBO	chest pain	NOC	Not related	Moderate	Recovered	None	Intermittent
36	ACTIVE	chest pain	NOC	Not related	Mild	Not yet recovered	None	Continuous
22	ACTIVE	palpitations	POR	Unlikely	Mild	Recovered	None	Intermittent
38	ACTIVE	chest pain and pain under left arm pit	POR	Unlikely	Mild	Recovered	None	Single Episode
55	ACTIVE	chest pain	POR	Not related	Mild	Recovered	None	Single Episode
42	PLACEBO	feeling of tachycardia	SMH	Possible	Mild	Recovered	None	Single Episode

*NOC = Nottingham City Hospital; POR = Portsmouth Hospitals NHS Trust; SMH = St Mary's Hospital, Imperial College Healthcare NHS Trust
†Action taken concerning study medication

Details of the serious adverse events are given in **Supplementary eTables 21 and 22**. There were 3 in the placebo group and one in the azithromycin group. All were related to the asthma exacerbations being studied and were considered unlikely or not related to study drug.

Supplementary eTable 21: Serious Adverse Events

Age (years)	Arm	Classification	Action taken	Event Description	Site	Relation to study drug	Severity
18	PLACEBO	Serious	Hospitalisation required	Pt became wheezy and short of breath, 13/10/12, presented to accident and emergency on 14/10/2012 and was admitted overnight. Diagnosis exacerbation of asthma.	GLA	Unlikely	Moderate
22	PLACEBO	Serious	Hospitalisation required	Exacerbation of underlying asthma. Admitted to Hospital at 9am on 7/Oct/2013 with extreme symptoms of breathlessness.	NNU	Not related	Severe
47	PLACEBO	Serious	Hospitalisation required	Acute exacerbation of asthma	A32	Not related	Moderate
49.	ACTIVE	Serious	Hospitalisation required	Shortness of breath and wheeze- non- infective exacerbation of asthma	A29	Not related	Moderate

Supplementary eTable 22: Serious Adverse Events continued

Frequency	Comments	Ongoing	Outcome	Category
Single Episode		No	Recovered	Respiratory, thoracic and mediastinal disorders
Unknown	Continuation of patients existing underlying condition. Classed as AE	No	Recovered	Respiratory, thoracic and mediastinal disorders
Single Episode	Admitted to hospital in Chester with asthma exacerbation for 3 nights.	No	Recovered	Respiratory, thoracic and mediastinal disorders
Single Episode	Patient was admitted with shortness of breath and kept in overnight	Yes	Not yet recovered	Respiratory, thoracic and mediastinal disorders

References

1. Bisgaard H, Hermansen MN, Bonnelykke K, et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *Bmj.* 2010;341:c4978.
2. Johnston SL, Blasi F, Black PN, Martin RJ, Farrell DJ, Nieman RB. The effect of telithromycin in acute exacerbations of asthma. *N Engl J Med.* Apr 13 2006;354(15):1589-1600.
3. Brusselle GG, Vanderstichele C, Jordens P, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax.* Apr 2013;68(4):322-329.

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Data Monitoring and Ethics Committee
An independent Data Monitoring and Ethics Committee (DMEC) was established to review adverse event reports and any ongoing safety issues. The DMEC membership is listed below:

Independent members
Professor Jonathan Grigg – Chair
Dr Stephen Bremner – Independent Statistician
Dr Peter Howarth – Independent Member

Trial Steering Committee
A Trial Steering Committee (TSC) was established to oversee the conduct of the study. The TSC membership is listed below:

Independent members
Professor Wisia Wedzicha – Chair
Professor Peter Calverley - Independent Member
Professor Ratko Djukanovic – Independent Member
Ms Leanne Metcalf, Asthma UK – Patient representative, Independent Member
Professor Mike Thomas – Independent Member

Non-members in attendance
Professor Deborah Ashby – Senior Statistician
Professor Chris Brightling – Principal Investigator, Leicester
Mrs Mary Cross – Operations Manager, Imperial Clinical Trials Unit
Professor Sebastian Johnston – Chief Investigator
Ms Laura Robison – Trial Manager (until February 2013)
Dr Zahid Sattar – Trial Manager (until April 2015)
Dr Jane Warwick – Senior Statistician (until June 2014)
Dr Alexina Mason – Junior Statistician (until Jan 2015)
Dr Ernie Wong – Research Fellow, Imperial College